L-DOPA for Parkinson's disease – a bittersweet pill

Lane E.L.

Cardiff School of Pharmacy and Pharmaceutical Sciences
Cardiff University
Redwood Building
King Edward VII Ave
Cardiff
Wales
CF10 3NB

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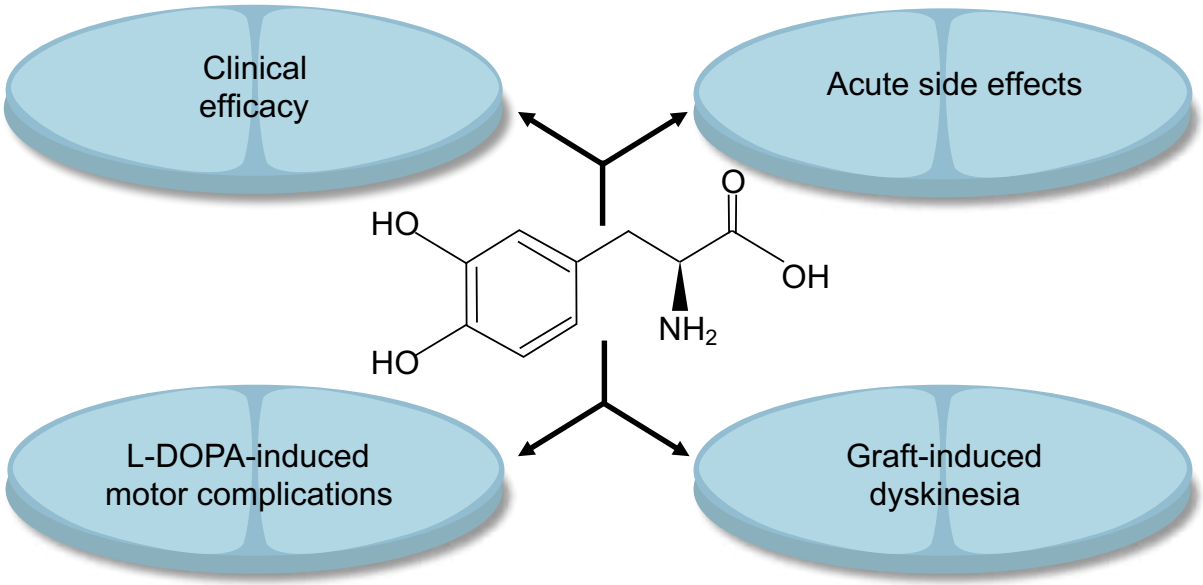

Abstract

3,4-dihydroxy-L-phenylalanine (L-DOPA) is the gold standard treatment for Parkinson's disease. It has earned that title through its highly effective treatment of some of the motor symptoms in the early stages of the disease but it is a far from perfect drug. The inevitable long term treatment that comes with this chronic neurodegenerative condition raises the risk significantly of the development of motor fluctuations including disabling L-DOPA-induced dyskinesia. Being unsurpassed as a therapy means that understanding the mechanisms of dyskinesia priming and induction is vital to the search for therapies to treat these side effects and allow optimal use of L-DOPA. However, L-DOPA use may also have consequences (positive or negative) for the development of other interventions, such as cell transplantation, which are designed to treat or repair the ailing brain. This review looks at the issues around the use of L-DOPA with a focus on its potential impact on advanced reparative interventions.

Introduction

At little over 200 years since James Parkinson's first description of the symptoms of 'the Shaking Palsy' (Parkinson, 1817), it is also 60 years since Arvid Carlsson's experiments showing the positive effects of the aromatic amino acid, dopamine precursor, 3,4-dihydroxy-L-phenylalanine (L-DOPA) in a rodent model of akinesia (Carlsson *et al.*, 1957). The reports that followed of early attempts to treat Parkinson's disease in patients with D/L-DOPA intravenously had varied outcomes (Birkmayer & Hornykiewicz, 1961). Persistence paid off, and the discovery that oral administration of D/L-DOPA, slowly titrated up, allowed much bigger, more clinically effective doses to be administered (Cotzias *et al.*, 1967). Two further refinements to the approach led to success; selectively administering the active isomer L-DOPA, and the co-administration with a peripheral amino acid decarboxylase inhibitor. These contributed to further minimising the side effects of peripheral conversion to dopamine and improved efficacy by enhancing passage across the blood brain barrier (Cotzias, 1968; Cotzias *et al.*, 1969a; b; Papavasiliou *et al.*, 1972). This provided a more consistent and sustained beneficial outcome with the management of the gastrointestinal side effects that had thwarted its use in earlier studies (Cotzias *et al.*, 1969b; Papavasiliou *et al.*, 1972). Revolutionising treatment for Parkinson's disease, L-DOPA rapidly became, and has remained, the 'gold standard' therapy for Parkinson's disease. Nevertheless, throughout this period, its optimal use has continued to be debated. There is no doubt in the remarkable ability of this drug to alleviate the motor symptoms of the disease and it is a vital cornerstone of current pharmacotherapy. Yet it is a drug with two sides, and ongoing controversies over the decades of its use have largely centred on the development of motor fluctuations which occur with high dose and/or long term therapy. Moreover, given that patients entering clinical trials will inevitably be on L-DOPA therapy, it is important that L-DOPA and its side effects are considered preclinically as factors in the search for new therapeutic or disease modifying strategies. This is exemplified in the case of foetal cell transplantation, in which patients developed an intractable form of dyskinesia as an apparent direct result of the surgically delivered reparative intervention, that has been linked to pre-treatment with L-DOPA. This review highlights the therapeutic effects of L-DOPA in the treatment of Parkinson's disease, moving on to discuss the issues of L-DOPA-induced complications. Importantly, the role L-DOPA and dyskinesia play in the context of

newly developed therapies will be emphasised and discussed as a consideration for future interventions.

L-DOPA in the clinical management of Parkinson's disease

The cardinal symptoms of Parkinson's disease are generally still recognised as those described by James Parkinson, bradykinesia/akinesia, resting tremor, postural instability and muscular rigidity (Parkinson, 1817). Prior to the development of L-DOPA, there was a very limited choice of medical interventions to alleviate any of these symptoms. The anticholinergic drugs, muscarinic receptor antagonists, are still available for use today but have limited ability to treat the motor symptoms, are troublesome to administer in the elderly and come with a host of cholinergic side effects. L-DOPA revolutionised the treatment of PD, improving the cardinal symptoms of rigidity and bradykinesia as well as functional disability although with more limited effects on tremor (Godwin-Austen *et al.*, 1969). These positive effects reinforced the hypothesis derived from the pathology of the disease, that many of the symptoms (although not all) were dopaminergic in origin (Bernheimer *et al.*, 1973). The addition of carbidopa, or latterly benserazide, as peripheral aromatic amino acid decarboxylase inhibitors reduced the dose required for beneficial effects and gave better control of motor symptoms, along with a reduction in the cardiovascular and gastrointestinal side effects.

L-DOPA is transported through the blood brain barrier on the large amino acid transporter 1 (LAAT1) where it is taken up into dopaminergic terminals and converted into dopamine by the enzyme aromatic amino acid decarboxylase. There are two components to the resulting clinical response, known as the short-duration and long-duration responses. The immediate, relatively short-lived (2-3 h) improvement in motor symptoms of the short-duration response parallels the plasma levels of L-DOPA. The longer duration response builds up over several days or even weeks, such that the maximal effects of L-DOPA can take several months. Following cessation of treatment, estimates in the decline of the long-duration response following L-DOPA withdrawal vary from days to weeks depending on the study (Muenter & Tyce, 1971; Fahn *et al.*, 2004). During the first 1–3 years of therapy the clinical response, measured as the ON-phase and dubbed the "honeymoon period", is relatively constant. It is likely that the long duration response in dominating through this

period and is thought to contribute to at least half the overall L-DOPA response (Nutt *et al.*, 1997; Fahn *et al.*, 2004). Quality of life is significantly enhanced from the use of L-DOPA as pharmacotherapy clinical benefit over dopamine agonists in symptom alleviation. In fact demonstrable L-DOPA responsiveness is such a defining feature of the disease that it forms a part of the diagnostic criteria (Postuma et al., 2015). As the disease advances responsiveness to L-DOPA declines but as doses increases, this is coincident with more rapid reductions in efficacy at the end of dose known as 'OFF-phase' (Fabbri *et al.*, 2016). A drug that has been used widely for 50 years nevertheless, questions still remain over its full mechanism of action beyond conversion to dopamine, as other metabolic products of L-DOPA have been shown to have possible glutamatergic activity, unrelated to the dopamine synthesis (Guatteo *et al.*, 2013; De Deurwaerdere *et al.*, 2017)

L-DOPA mediated toxicity

The question as to whether L-DOPA treatment could impact on disease progression or mortality arose very early in its clinical use. Given that L-DOPA can be converted to highly reactive semiquinone and quinone derivatives, and that the metabolism of the dopamine that is produced could contribute to oxidative stress through the Fenton reaction (Smith *et al.*, 1994), there was a concern over neurotoxicity with several early studies seeking to determine whether L-DOPA itself may contribute to disease pathophysiology (Barbeau & Roy, 1976; Rajput *et al.*, 1997; Melamed *et al.*, 1998). Certainly *in vitro* determination of cellular toxicity illustrated that L-DOPA and dopamine can be detrimental to cell survival (Ziv *et al.*, 1997; Mytilineou *et al.*, 2003), but *in vivo* studies did not consistently support this finding (Camp *et al.*, 2000; Datla *et al.*, 2001; Ferrario *et al.*, 2003; Mytilineou *et al.*, 2003). As a result, and with inconclusive clinical attempts to address this, the clinical relevance of the preclinical findings was in question. Eventually a large clinical cohort study, ELLDOPA, found no evidence of an impact of L-DOPA on disease progression (Fahn *et al.*, 2004).

Other studies focused on the role of L-DOPA in mortality in Parkinson's disease, the most recent of which have not demonstrated any reduction in mortality directly associated with the use of L-DOPA, although they concomitantly highlight limited benefit (Di Rocco *et al.*, 1996; 1998; Hely *et al.*, 1999; Montastruc *et al.*, 2001; Rascol *et al.*, 2006). In those studies that do show improvements in mortality rates, the focus is largely on early stage,

uncomplicated PD, suggesting that the most benefit is seen during the 'honeymoon period' (Matarazzo *et al.*, 2018). Overriding factors that do increase mortality rate, determined in long running studies of up to 15 years, are unrelated to medication and pertain to incidence of dementia and the age of onset of the disease (Hely *et al.*, 1999; Montastruc *et al.*, 2001).

L-DOPA mediated complications

Acutely L-DOPA causes side effects of orthostatic hypotension, excessive daytime somnolence and nausea. These are generally well tolerated by patients but as the disease advances the use of L-DOPA becomes more complex; some symptoms become resistant to the treatment and motor fluctuations are a major feature of the clinical management. The phenomena of freezing, wearing off and L-DOPA induced dyskinesia (LID) as side effects of long term, high dose L-DOPA use were recognised as very early on in its clinical use (Cotzias *et al.*, 1969b; Barbeau, 1971). Although the fluctuations between ON and OFF states, are distressing and disabling, the treatment limiting side effect of L-DOPA is the abnormal involuntary movements known as LID. LID occur as choreic and/or dystonic movements; chorea being abnormal, purposeless, non-rhythmic, rapid movements commonly affecting the neck and limbs while dystonia are sustained contractions of muscles, which may be localised to specific muscle groups. LID appear as either peak dose or diphasic/end-of-dose dyskinesia which have distinct temporal profiles in relation to each dose of L-DOPA. Most troublesome are the peak dose dyskinesia, occurring during the peak plasma concentration of L-DOPA, while the diphasic dyskinesia occur at low L-DOPA levels at the start and end of the dose of L-DOPA.

A retrospective cumulative literature synthesis of data on the frequency of LID development across clinical trial cohorts, found that within the first year of treatment very few patients experienced dyskinesia (Ahlskog & Muenter, 2001). They identified a frequency of dyskinesia of around 40% by 4-6 years of L-DOPA treatment, reaching 90% within 9 years of L-DOPA treatment with a similar profile for motor fluctuations (Ahlskog & Muenter, 2001). Although a view not consistently held, with the more widespread use of dopamine agonists in the 1980s and 1990s came the trend for delayed use of L-DOPA in favour of these alternatives. The principle was that by sparing the use of L-DOPA, the onset of LID would be delayed. However, a prospective study comparing initial treatment with either the

dopamine agonist ropinirole or L-DOPA confirmed that although dyskinesia incidence was less on ropinirole, once L-DOPA therapy commenced there was no beneficial delay in dyskinesia onset (Rascol *et al.*, 2000; Hauser *et al.*, 2007). Similar findings were reported in the long running PDRG-UK following patients initially randomised to L-DOPA, L-DOPA plus selegiline or the dopamine agonist bromocriptine (Katzenschlager *et al.*, 2008). Dopamine agonists also carry the risk of impulse control disorders and are less effective at alleviating symptoms particularly at later stages of the disease. Current guidance is that low dose L-DOPA should be considered as an option from the onset of pharmacotherapy alongside dopamine agonists and monoamine oxidase B inhibitors.

Risk factors for L-DOPA induced dyskinesia

Evidence that LID are related predominantly to the severity of striatal dopaminergic denervation comes from multiple avenues. Patients affected by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) who had experienced rapid and severe nigrostriatal denervation, developed similarly rapid LID onset after L-DOPA treatment was initiated (Langston & Ballard, 1984; Ballard *et al.*, 1985). Moreover, with PD often being an asymmetric disorder, the side of the body most affected by the disease is likely to be the first to be affected by the LID (Ruzicka *et al.*, 2005). Disease duration is a major risk variable as is dose of L-DOPA but both are linked to disease severity, L-DOPA dose being higher in more severely affected patients (Cilia *et al.*, 2014). Rodent and primate studies support these findings, demonstrating that LID following chronic L-DOPA are only produced in animals with severe denervation but highlight that this is not the only determinant (Di Monte *et al.*, 2000; Guigoni *et al.*, 2005; Lindgren *et al.*, 2007).

There are other identified risk factors for LID, with a strong association with age at onset of Parkinson's, with younger patients (roughly defined as under 50 at disease onset) having an increased risk (Warren Olanow *et al.*, 2013; Zhang *et al.*, 2013), although this may be explained by factors other than age *per se* including that earlier onset of disease is linked to higher degree of dopamine denervation at disease onset, higher dopamine turnover and enhanced dopamine receptor signalling (de la Fuente-Fernandez *et al.*, 2004; Sossi *et al.*, 2006; Troiano *et al.*, 2009). The akinetic-rigid Parkinson's phenotype has been linked to higher risk over the tremor dominant phenotypes but is again linked to more severe striatal

dopaminergic denervation (Zhang *et al.*, 2013). Weight and gender have been identified as risk factors in some studies but the evidence is less consistent (Sharma *et al.*, 2010; Hassin-Baer *et al.*, 2011; Warren Olanow *et al.*, 2013). Genetics variants in the dopaminergic machinery are also associated with altered LID risk, including genes encoding dopamine receptors, the dopamine transporter, catechol-o-methyl transferase, and monoamine oxidase (Comi *et al.*, 2017; Kusters *et al.*, 2018; Sampaio *et al.*, 2018).

Patients themselves have a mixed relationship with L-DOPA for these reasons. Caution on behalf of the medical profession, combined with patients reluctant to increase their dose in attempts to minimise the onset of LID, may lead to patients not receiving the full benefit they could have from L-DOPA. However, it has been reported that once patients reach this stage and experience dyskinesia, they find it more acceptable than bradykinesia and reduced efficacy of lower doses (Hung *et al.*, 2010; Hattori *et al.*, 2012). The impact of LID on quality of life is generally negative but varies from study to study (Pechevis *et al.*, 2005; Rahman *et al.*, 2008; Montel *et al.*, 2009; Politis *et al.*, 2010b), but in addition they are associated with increased risk of falls and depression (Ashburn *et al.*, 2001; Pechevis *et al.*, 2005) .

Pathophysiology of L-DOPA–induced dyskinesia

In early disease, the few intact dopaminergic neurons remaining are still able to store dopamine, and regulate its release through the feedback dopamine D_2 autoreceptors in the pre-synaptic membrane. This, along with some remaining dopamine transporter activity limits excessive post-synaptic DA receptor stimulation. However, as dopaminergic terminals are lost through disease progression (Vermeulen *et al.*, 1995), the short duration response begins to dominate with greater fluctuations in function as plasma levels of L-DOPA peak and trough. With the reduced ability of dopaminergic terminals to convert L-DOPA to dopamine, L-DOPA handling and the subsequent synthesis and regulation of dopamine in the striatum is now occurring in non-dopaminergic 5-HT terminals which also contain the required synthesis machinery (aromatic amino acid decarboxylase), but lack the regulatory controls (D2 autoreceptors) (Carta & Tronci, 2014). Imaging data indicates that there is in fact increased 5-HT striatal innervation in dyskinetic patients, as opposed to those who have a stable response to L-DOPA, both greater pallidal serotonergic innervation and greater 5-

HT transporter/dopamine transporter ratios (Politis *et al.*, 2014; Smith *et al.*, 2015; Roussakis *et al.*, 2016) indicating a significant role for the serotonin system in LID. The lack of dopaminergic autoreceptors on the 5-HT terminals leads to abnormal presynaptic dopamine handling and thereby dysregulated dopamine release.

An additional key feature of LID is that they become intractable. Once LID are established the relationship to dose of L-DOPA becomes less linear and L-DOPA holidays have no significant benefit in reducing LID clinically. This priming effect is mirrored in both MPTP-treated non-human primates and 6-OHDA-lesioned rodents treated chronically with L-DOPA. Both models have severe denervation of the nigrostriatal pathway and chronic L-DOPA administration leads to the development of abnormal involuntary movements likened to LID phenomenologically and biochemically (Bedard *et al.*, 1986; Clarke *et al.*, 1987; Doucet *et al.*, 1996; Cenci *et al.*, 1998; Cenci *et al.*, 1999). Pharmacologically the LID generated are responsive to amantadine as seen in clinical settings (Blanchet *et al.*, 1998; Lundblad *et al.*, 2002). These models serve as useful exploratory tools to understand the mechanisms underlying LID and in the search for new treatment strategies which would allow L-DOPA to be utilised to its full potential throughout the disease (reviewed in this volume Fox and Brotchie 2018). With the use of these models, alongside the growing knowledge of clinical risk factors and how this might relate to the pathophysiology of the condition, a picture is growing of the long-term changes that evolve during chronic L-DOPA administration and temporally associate with LID induction and severity. The fluctuations in striatal dopamine levels caused by serotonergic handling of the L-DOPA have consequences for the control of post-synaptic medium spiny neurons: excitatory D_1 dopamine receptor containing medium spiny neurons of the direct basal ganglia pathway or the inhibitory D_2 expressing cells of the indirect pathway (see figure 1). Originally described by Albin et al 1989, the basal ganglia circuitry is affected by the loss of dopamine in the disease, in which the dysregulation of the output nuclei (globus pallidus interna and substantia nigra pars reticulata) leads to reduced motor cortex output. L-DOPA induced dyskinesia arise through the excessive activity at the output nuclei disinhibiting the thalamus leading to excessive cortical stimulation (see figure 1, this is excellently and extensively reviewed elsewhere (Bastide *et al.*, 2015; Cenci *et al.*, 2018).

Dopaminergic receptor dysregulation is heavily evidenced, as are the underlying ERK and cAMP pathways (Westin *et al.*, 2001; Westin *et al.*, 2007; Lebel *et al.*, 2010; Park *et al.*, 2014) Studies of these pathways alongside recent chemogenetic manipulation of specific cell types supports the notion that altered dopaminergic activity at the D_1 and D_2 receptors drives the abnormal movements (Aubert *et al.*, 2005; Gold *et al.*, 2007; Guigoni & Bezard, 2009; Alcacer *et al.*, 2017). Immediate early gene expression in the postsynaptic medium spiny neurons is elevated following chronic L-DOPA administration, extracellular signal-related protein kinase (ERK) and mitogen and stress activating factor-1 (MSK1) are involved in the increased expression of ΔfosB, which is highly correlated to LID expression in rodent and primate models (Andersson *et al.*, 1999; Cenci *et al.*, 1999; Valastro *et al.*, 2007; Lindgren *et al.*, 2011). This is a long-term upregulation which appears to be causally related to LID expression and intractability, with functional inactivation reducing dyskinesia in the rodent model whilst conversely overexpression of its dimerising partner JunD has the same effect (Berton *et al.*, 2009; Feyder *et al.*, 2016).

Long term L-DOPA treatment and the development of LID also cause other alterations to basal ganglia circuitry (Rylander Ottosson & Lane, 2016). The corticostriatal synapse between the glutamatergic cortical efferent and striatal MSNs is an essential pathway for improvement in motor function after L-DOPA administration. Both long term potentiation (LTP) and long term depotentiation (LTD) are key features of excitatory corticostriatal synapses where they are believed to underlie motor-skilled learning, cognitive performance and reward mechanisms (Calabresi *et al.*, 2007). In rats with dopaminergic denervation, both LTP and LTD are absent or severely altered (Picconi *et al.*, 2003; Paille *et al.*, 2010) and restoration of LTP has been seen in the dorsolateral striatum with repeated L-DOPA administration (Picconi *et al.*, 2003). In both rodent models and in patients with LID, aberrant corticostriatal plasticity has been demonstrated (Morgante *et al.*, 2006; Calabresi *et al.*, 2007). In the rat PD model, LID is associated with an irreversible LTP of the striatal medium spiny neurons, which fail to depotentiate (Picconi *et al.*, 2003) and also show persistent deficits in LTD (Picconi *et al.*, 2011). Clinically, LTP-like plasticity been detected in the motor cortex which was normalised by non-LID inducing L-DOPA treatment (Morgante *et al.*, 2006) indicating that similar mechanisms may occur in patients. This plethora of

changes resulting from L-DOPA treatment has a consequence for future interventions which seek to restore the neuronal circuitry.

L-DOPA and cell transplantation

Developing at a much slower rate, but with the research embedded in the same origins as the use of L-DOPA as a dopamine replacement strategy, a longer term, improved supply of striatal dopamine has been explored. The principle of cell transplantation in Parkinson's disease is to implant cells into the dopamine-depleted striatum to act as a dopamine supplier, replacing the lost neurotransmitter and therefore restoring function. Initial rodent studies demonstrated the feasibility of this approach, using either catecholamine producing adrenal medulla tissue or dopaminergic neurons of the developing foetal brain ventral mesencephalon (Perlow *et al.*, 1979; Björklund *et al.*, 1983; Freed, 1983). After much practical refinement of the approaches, both reached open label clinical trials in the late 1980's (Backlund *et al.*, 1985; Goetz *et al.*, 1989; Kelly *et al.*, 1989). Although ethically compromised, the most clinically successful approach was the use of early stage foetal ventral mesencephalic cells to the extent that several small open label studies and two US government funded double blind trials were delivered (Lindvall *et al.*, 1990; Spencer *et al.*, 1992; Molina *et al.*, 1994; Kopyov *et al.*, 1996; Kopyov *et al.*, 1997; Levivier *et al.*, 1997; Freed *et al.*, 2001; Hagell & Brundin, 2001; Olanow *et al.*, 2003).

Although variable, some very successful outcomes were reported in the open label trials; improvement in UPDRS and reduction in 'off' time demonstrated some direct functional benefit with increases 'on' time in the absence of dyskinesia indicative of improved dopamine handling (Lindvall *et al.*, 1990; Lindvall *et al.*, 1992; Freeman *et al.*, 1995; Kopyov *et al.*, 1997). One of the best predictors for a positive outcome is indeed a positive response to L-DOPA prior to transplantation (Freed *et al.*, 2004). Unfortunately, the double blind clinical trials were disappointing overall although benefit was identified when patients were stratified by age (Freed *et al.*, 2001; Olanow *et al.*, 2009). Some individuals have, over time, significantly reduced or even ceased their L-DOPA medication for a sustained period of several years before returning back to the therapy (Kefalopoulou *et al.*, 2013).

Graft-induced dyskinesia

Whilst the variability in post-transplantation outcomes was concerning, the first report of the double-blind studies raised a major issue, detailing a motor side effect now known as graft-induced dyskinesia (Freed *et al.*, 2001). At one year follow-up assessments, 5 out of 33 grafted patients, all of whom had improved functionally, presented with involuntary movements. Predominantly dystonia in the head and upper extremities, these movements persisted for several days after cessation of L-DOPA, in the clinically defined OFF-phase. All patients were under the age of 60 at the time of transplantation and had pre-existing LID in response to their ongoing Parkinson's medication (Freed *et al.*, 2001). In a retrospective review of video footage of 14 patients who had collectively received foetal cell transplants (Hagell *et al.*, 2002), the Swedish team identified mild graft-induced dyskinesia in 8 patients but a further 6 were considered to present with moderate, graft-induced dyskinesia with 2 presenting with a clinically relevant problem. These persisted for up 9 weeks following withdrawal of L-dopa. Phenotypically the movements were distinct from the Denver/Colombia trial, with mixed effects on dystonia but hyperkinesia increased in all patients and choreiform movements including brief dystonic posturing alongside repetitive stereotypic or ballistic movements. In the second of the double-blind studies, Olanow and colleagues reported stereotypic, rhythmic graft-induced dyskinesia in 56% (13 out of 23) of transplant recipients, 3 of whom required additional intervention due to their severity (Olanow *et al.*, 2003). These additional interventions were varied with mixed degrees of success which may give some indications as to the pathophysiology but with very limited numbers of patients and limited information in the public domain this vague at best (reviewed in (Lane *et al.*, 2010). Typically deep brain stimulation (DBS) of either the subthalamic nucleus (STN) or the globus pallidus interna (GPi) was attempted with STN DBS more commonly reported as successful but no conclusions can really be drawn (Freed *et al.*, 2001; Ma *et al.*, 2002; Cho *et al.*, 2005; Herzog *et al.*, 2008; Beaulieu-Boire & Fasano, 2015)(see figure 1D).

At the discovery of repeated incidence of graft-induced dyskinesia, no further clinical trials were initiated and the imperative was to understand the mechanisms underlying this unprecedented side effect. There is no evidence that any side effects of this nature were anticipated form the preclinical work. Two reasons could underlie this; either that spontaneous dyskinesia were simply not expressed, or that they were, had not observed or

reported. The majority of transplantation studies were, and continue to be, performed in the 6-OHDA lesioned rodent, a unilaterally lesioned model in which syngeneic foetal grafts are easily produced. Dopaminergic restoration is readily monitored using both hand testing for determinants of motor function and drug-induced rotational responses for objective assessment (reviewed in (Lane, 2011). MPTP-treated primate studies were also conducted but logistically are far more complex to perform foetal grafting. As a contextual aside, the methodology for evaluating LID in the MPTP treated primate model was well established at this time, and considered very clinically translatable (Clarke *et al.*, 1987; Crossman *et al.*, 1987). In contrast, whilst stereotypy scoring was well established in the rat, a representation of dyskinesia in the 6-OHDA lesioned rat had only recently been published by Cenci (1998) and was still not widely accepted as a clinical correlate, although it is now a proven and well-established model. The three clinical trials reporting graft-induced dyskinesia varied in many aspects (surgical trajectory, numbers of cells transplanted, form of the transplanted cells, storage of the transplanted cells prior to transplantation, immunosuppression post transplantation (Lane, 2011), but one key commonality was that all patients had pre-existing L-DOPA-induced peak dose dyskinesia. In addition, patients would continue to require dopaminergic pharmacotherapy following transplantation to a greater or lesser extent as the cells matured. Only a small number of patients successfully withdrew their anti-parkinson's treatment completely as a result of cell transplantation (Kefalopoulou *et al.*, 2013).

The role of L-DOPA in graft-induced dyskinesia

Prior to the realisation of graft-induced dyskinesia, the only studies to combine cell transplantation and L-DOPA were designed to address two very specific questions. The first was the potential toxicity of L-DOPA on the grafted cells and, similar to the toxicity studies described above, while *in vitro* toxicity was demonstrated (Alexander *et al.*, 1997), the more relevant *in vivo* studies were less conclusive. Blunt and colleagues showed that treatment of rats with high doses of L-DOPA post-graft did not have a detrimental effect on graft survival or functional activity (as measured by amphetamine and apomorphine-induced rotations) (Blunt *et al.*, 1992). Conversely, other investigators identified impaired cell survival and morphological development of these embryonic dopaminergic neurons (Steece-Collier *et al.*, 1990; Yurek *et al.*, 1991) which was thought to be driven by dopamine production rather

than direct effects of L-DOPA (Alexander *et al.*, 1997). Prior to clinical trials only a single animal study explored whether grafts alleviate LID showing a clear reduction in L-DOPA mediated movements and improved regulation of dopamine (Lee *et al.*, 2000). However, no study combined these two questions and determined the effects in the morphology and function of the graft in a clinically relevant paradigm, i.e. L-DOPA administration with LID generation and evaluation prior to transplantation, with ongoing L-DOPA treatment following the procedure and examination of any dyskinesia unrelated to L-DOPA.

Following the revelation of graft-induced dyskinesia, a close examination of freely moving 6-OHDA lesioned rats, with established LID at the time of transplantation with syngeneic rat ventral mesencephalon, showed the induction of spontaneous dyskinesia. The incidence of the movements was higher in those with larger grafts, and consisted of pronounced axial twisting contralateral to the lesioned side and forelimb hyperkinesia (Lane *et al.*, 2006). However, these were generally mild, and were not evoked in later test sessions, possibly due to familiarity with the process and reduced stress. Two, more consistent approaches were determined, although both induce abnormal movements through pharmacological challenge. During standardised amphetamine-induced rotation assessment of graft function, dyskinetic movements were seen to emerge over time post-transplantation, behaviours not seen following amphetamine in animals without grafts. Importantly, these amphetamine-induced behaviours were more prominent in L-DOPA treated animals and correlated with the severity of pre-existing LID and inversely related to the improvement in post-transplantation LID (Carlsson *et al.*, 2006; Lane *et al.*, 2006; Lane *et al.*, 2009). The second approach was a close examination of the effect of transplantation on LID and despite global improvement, an altered profile of LID has been reported post transplantation, with an increase in head movements and forelimb hyperkinesia. The paradox emerges as both studies replicated earlier findings of improved LID, simultaneous with the emergence of the novel, graft-induced behaviours. Indeed, LID reduction occurs relatively early post-transplantation compared to measures of efficacy of the graft on motor function, suggesting that even immature transplanted neurons are able to handle exogenous L-DOPA in preference to the striatal 5-HT terminals. The consequence of this improved dopamine handling appears to be a gradual normalisation of the post-synaptic hypersensitivity to dopamine with normalisation of fosB/ΔfosB levels and improved apomorphine-induced

rotations (used as an indication of post-synaptic dopamine receptor sensitivity) (Lane *et al.*, 2009) i.e. normalisation of the processes that mediate LID. The driver underlying these abnormal behaviours is therefore remains unclear.

Neither of the rodent models gives a true representation of graft-induced dyskinesia and until recently, no studies had reported transplantation studies in MPTP-treated primates with prior established LID, likely to be the closest translational model to the clinical situation. A large MPTP-treated primate study comparing focal and widespread grafts dopaminergic and non-dopaminergic grafts, with and without L-DOPA prior LID generation, confirmed reduced LID following transplantation. Frustratingly, no spontaneous graft-induced dyskinesia were observed in this study designed to determine whether L-DOPA priming was a key risk factor, thus leaving the search ongoing for the root cause (Kordower *et al.*, 2017). However, L-DOPA treatment was not continued post-transplantation, and therefore it is impossible to determine whether ongoing L-DOPA following transplantation would impact on grafted cell phenotype, innervation or graft-induced dyskinesia.

The effect of L-DOPA on the maturation and survival of transplanted cells was evaluated in a small number of studies prior to clinical trials but this has been revisited since the revelations of graft-induced dyskinesia. Comparing initiation of L-DOPA treatment from either 5 weeks pre or 9 weeks post-transplantation found that pre-exposure to chronic L-DOPA had no effect on cell survival significantly reduced both behavioural and neurochemical efficacy of embryonic dopamine grafts (Steece-Collier *et al.*, 2009). A second study compared pre- and post-transplantation treatment with L-DOPA with two graft types, an allogeneic vs xenogeneic graft. While graft integrity was unaffected by L-DOPA, inflammatory responses were higher if L-DOPA was administered with a less immunologically compatible graft (mouse derived ventral mesencephalon) (Breger *et al.*, 2017). As in the previous rodent studies, the MPTP non human primate study also demonstrate that an L-DOPA primed striatum is not a more aversive environment for transplanted foetal cells with no difference in transplant survival between L-DOPA primed and L-DOPA naïve primates (Steece-Collier *et al.*, 2009; Breger *et al.*, 2017; Kordower *et al.*, 2017).

However, overall we still do not have model representation of true graft-induced dyskinesia, perhaps due to over-simplification of the models, Graft-induced dyskinesia is unlikely to be driven by a single factor, using multi-factorial approaches may be necessary to determine whether there is a clinically meaningful effect of L-DOPA on grafts. The role of endogenous and exogenous 5-HT innervation from the host or within the graft has been the focus of a lot of speculation in relation to both LID and graft-induced dyskinesia. A detailed discussion is beyond the scope of this review and is discussed elsewhere (see (Shin *et al.*, 2012a; Niccolini *et al.*, 2014), but in brief, there is a high degree of endogenous striatal serotonergic innervation which is enhanced following foetal cell transplantation. While there is strong evidence that L-DOPA management through the endogenous striatal innervation contributes to dysregulation of L-DOPA handling and thus LID (Carta & Björklund, 2018), the picture in relation to graft-induced dyskinesia is less convincing. Clinical imaging evidence of high striatal 5-HT transporter density in transplanted patients with graft-induced dyskinesia is assumed to arise from 'contamination' of the graft material by the neighbouring dorsal raphe (Politis *et al.*, 2010a; Politis *et al.*, 2011). Upon administration of the 5-HT_{1A} receptor antagonist buspirone, grafted patients showed reduced expression of the induced dyskinesia, although this could be explained by the dopamine D_2 receptor partial agonist activity of the drug leading to antagonist actions in high dopamine areas (Politis *et al.*, 2010a; Shin *et al.*, 2012b). Importantly, in the study by Politis and colleagues (2010a), no patient with a graft without the side effect underwent scanning as a comparator and post mortem data from a Canadian transplanted cohort showed high striatal 5-HT transporter content in the absence of graft-induced dyskinesia (Mendez *et al.*, 2005). The aforementioned reduction in dyskinesia with buspirone was not reproduced in a patient from a different transplanted cohort (Beaulieu-Boire & Fasano, 2015).

A detailed phenotypic analysis of grafts matured in the presence of L-DOPA administration is yet to be conducted, an excellent study of synapse formation has been performed producing interesting insights (Soderstrom *et al.*, 2008). Dopamine depletion in the striatum and LID generation cause loss of spines on the medium spiny neurons, the site of normal synapse formation with the dopaminergic nigrostriatal innervation. Transplanted neurons appear to form asymmetric synapses on the soma as a result, and these have been linked to

abnormal post-transplantation responses to L-DOPA (Soderstrom *et al.*, 2008). Dendritic spine loss occurs via dysregulation of intraspine Cav1.3 L-type Ca(2+)channels and can be prevented, in animal models, by administration of the calcium channel antagonist, nimodipine. When this was administered maintained spine integrity led to improved motor performance and reduction in L-DOPA-induced abnormal movements which supports the concept that the emergent abnormalities following transplantation in response to L-DOPA result from incomplete reinnervation rather than aberrant neuronal activity (Soderstrom *et al.*, 2010). Clearly the ability of the transplanted neurons to reform the deficit neuronal circuitry is compromised by L-DOPA, but the clinical implications of this remain unclear.

To date, the majority of preclinical animal studies exploring the interplay between cells and drug treatments have utilised transplantation of primary ventral mesencephalon. The next evolution of this work must be to examine the impact of L-DOPA and dopaminergic drugs on the transplantation of cells from other sources, primarily the human embryonic stem cells that will be used in upcoming clinical trials in the US (NYSTEM) and Europe (STEM-PD) (Kirkeby *et al.*, 2017; Studer, 2017). Stem cells carry dopamine receptors throughout differentiation. Both dopamine and 5-HT are known regulators of neurogenesis both *in vivo* and *in vitro* (Takamura *et al.*, 2014), and as such clearly have the potential to regulate the proliferation and differentiation of implanted stem cells. Dopamine receptors, in particular, the D2-like receptor family, are expressed at all stages of dopaminergic differentiation of H9 stem cells and dopamine agonists have been shown to alter proliferation, phenotype and neurite outgrowth in vitro (Belinsky *et al.*, 2013). *In vitro* exposure to dopamine late in the differentiation process (around the time cells might be transplanted) caused a dopamine D_2 receptor dependent increase in TH-positive clusters of cells and doubling of neurite formation (Belinsky *et al.*, 2013). This work is complicated by the need to transplant xenogeneically, to study the human cells in rats and although there are some strategies to facilitate this (the use of immunosuppression, immunodeficient rats or neonatal densensitisation) each has its problems. Inflammation may be an important cofactor to be considered alongside L-DOPA but it unclear whether this is feasible in a rodent model in a clinically relevant fashion. Both NYSTEM and STEM-PD trials indicate that due to the 'first in man' nature of these studies they are likely to be recruiting patients with established moderate Parkinson's disease are therefore likely to have pre-existing LID (Kirkeby *et al.*,

2017; Studer, 2017). The outcomes of TransEUro, a clinical study designed with the improved knowledge around graft-induced dyskinesia (Moore *et al.*, 2014), and of these new stem cell trials themselves will be hugely enlightening.

The future of L-DOPA

Despite its array of problems, L-DOPA has still not been bettered as a symptomatic treatment. There is still traction, therefore, in circumventing the acute and/or chronic side effects through improved delivery systems. One new oral approach has been approved, IPX066, which is an extended release L-DOPA/carbidopa capsule formulation (Hauser *et al.*, 2018). Oral therapy has highly variable gastrointestinal absorption leading to vast differences in plasma levels from dose to dose, some of which may be attributable to delayed gastric emptying and other gut abnormalities. There is currently the option, in severe disease with motor complication, to administer the drug through an intrajejunal route using the DuoDopa pump. This has been shown to have sustained beneficial effects on both motor and non-motor symptoms using an L-DOPA/carbidopa gel formulation. A trigel formulation with the addition of entacapone is currently in development, which may add increased clinical benefit (Senek *et al.*, 2017). New formulations in clinical trials currently include inhaled (CVT-301) (Lipp *et al.*, 2016) for management of 'OFF-phase' and liquid for subcutaneous administration (ND0612) as a patch-pump. ND0612 is being developed for moderate and severe disease and could produce much improved control of plasma L-DOPA levels and therefore dyskinesia (Freitas *et al.*, 2016; Ramot *et al.*, 2017).

Conclusions

L-DOPA is a high effective drug that has been widely used for over 50 years and will continue as the mainstay of Parkinson's disease therapy for many years to come. New formulations may extend its lifetime still further and hopefully will help manage some of the treatment-limiting side effects that are so problematic in advanced disease. Despite its longevity, it is clear that there is much we do not understand about the mechanisms underlying both the therapeutic and negative effects. Neuronal circuitry and function is altered through chronic L-DOPA treatment and while cell transplantation teaches us that this can be reversed, this opens up new questions about alternative pathways that might be generating motor side effects. The lessons from the cell transplantation experience is that,

at the very least, we must be alert to the potential consequence of L-DOPA and the consequent dyskinesia in novel reparative and neuroprotective strategies and consider how we factor this in to preclinical models.

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Figure 1

Illustration of postulated basal ganglia physiology and pathophysiology in Parkinson's disease, L-DOPA induced dyskinesia and graft-induced dyskinesia. Described originally by (Albin *et al.*, 1989), this figure illustrates the dopaminergic nigrostriatal innervation of the GABAergic medium spiny neurons (MSNs) of the striatum. These MSNs form two output pathways; direct pathway directly innervating the GPi/SNr output nuclei under the control of D1 dopamine receptors; indirect pathway innervating the GPe under the control of the D2 dopamine receptors. The indirect pathway then regulates the GPi/SNr via the STN. GABAergic regulation of the thalamus by the output nuclei then regulates cortical activity and motor function (many reciprocal pathways exist and have not been shown for clarity). B) Cortical control is disrupted by the loss of nigrostriatal dopamine in Parkinson's disease, the consequences of which is increased inhibitory input to the thalamus and lowered cortical excitation reducing movement. C) L-DOPA-induced dyskinesia arise through the excessive activity at the GPi/SNr disinhibiting the thalamus leading to excessive cortical stimulation. D) While it is evident there is excessive cortical drive, there is still no clarity about the activity of the basal ganglia nuclei during graft-induced dyskinesia given that for many patients it occurs simultaneous with improved function (although not necessarily).

(STN= subthalamic nucleus, GPi – globus pallidus interna, GPe – globus pallidus externa, SNr – substantia nigra pars reticulata, SNc – substantia nigra pars compacta

Abbreviations:
6-OHDA - 6-hydroxydopamine
DBS - deep brain stimulation
L-DOPA - 3,4-dihydroxy-L-phenylalanine
LID - L-DOPA-induced dyskinesia
MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
SNc – substantia nigra pars compacta
STN - subthalamic nucleus